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Solvent Partition for Terpenoid Rich Fraction From Crude Extract of *Eurycoma longifolia*

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ABSTRACT

The root of *Eurycoma longifolia* or locally called as Tongkat Ali contains numerous health benefits, mainly due to the bioactive compounds. The main compound of interest is from the group of quassinoids which is also known as degraded triterpenoids such as eurycomanone, eurycomalactone, longilactone, pasakbumin B and pasakbumin C. Quassinoids or degraded triterpenoids were reported to possess anti-malarial, anti-diabetic, anti-bacterial and anti-cancer effects. Therefore, it is important to concentrate terpenoids rich fraction from the crude extract of *E. longifolia* roots to enhance the reported bioactivities. The present study was carried out to determine the suitable solvent in concentrating terpenoids from the crude extract of *E. longifolia* roots. Organic solvents such as chloroform, butanol and ethyl acetate were selected to maximally recover terpenoids based on the principle of liquid-liquid extraction. The quality of the fractions was compared and analysed for the phytochemical composition. The results showed all organic fractions contained higher total terpenoid content than its aqueous counterpart. The chloroform fraction exhibited the highest yield of mass fraction (72.90 ± 0.15%) and the highest total terpenoid content (0.47 ± 0.01 mg OAE/L).

Keywords: *Eurycoma longifolia, terpenoids, liquid-liquid extraction*

1. INTRODUCTION

Eurycoma longifolia (Tongkat ali) is one of the famous traditional tropical plants in Malaysia. There are four different species, namely *E. longifolia*, *Entomophthora apiculata*, *Polyathia bullata* and *Goniothalamus* sp. [1]. However, *E. longifolia* is the most commonly used species for medicinal purposes. *E. longifolia* was proven to possess medicinal and healing properties. It was reported that the extract of *E. longifolia* possess anti-malaria [2-4], anti-inflammatory [5], anti-cancer [6-12], anti-microbial, anti-pyretic agents and aphrodisiac properties [13-15]. *E. longifolia* extract was also found to restore hormone imbalance and reduce cortisol or stress hormone [16]. Previous research reported that *E. longifolia* exhibited anti-diabetic effect [17-21]. The root extract of *E. longifolia* was also reported to reduce cholesterol and uric acid level [19, 22].

There roots and stems of *E. longifolia* contained different groups of phytochemicals such as alkaloids, phenolics, flavonoids, terpenoids or quassinoids, saponins, and proteins [15]. Quassinoids, alkaloids, flavonoids from *E. longifolia* extracts were reported to exhibit anti-malarial activity [6, 23-25]. Eurycomanone which is the marker compound of *E. longifolia* belongs to quassinoids or degraded triterpenoids was also reported to reduce inflammation and possess analgesic effects [8, 26, 27]. Eurycomanone was also reported to have anti-cancer activity on breast, lung and cervical cancer cells [7-8, 12, 28]. Phenolics such as quercetin, rutin and ellagic acid were reported to reduce erectile dysfunction [29].

Terpenoids have a wide range compounds with different polarities. The polarity differs mainly based on the functional groups such as aryl and acyl (polar), structure (linear or cyclized hydrocarbons, non-polar) and the addition of hydroxyl or methyl group (less polar) from its isoprene based skeleton. The volatility of terpenoids also alters the polarity of the compound. Small chain terpenoids (< 15 carbons) are usually volatile and less polar, whereas non-volatile terpenoids are non-polar and involve a very non-polar solvent like hexane (absolute or combined with other non-polar organic solvents) in its isolation process [30].

Previous studies reported many extraction methods to prepare crude extract from the stems, roots and leaves of *E. longifolia*. The methods were reflux, maceration and Soxhlet methods. Several organic solvents such as methanol, petroleum ether, butanol, dimethyl ether, dichloromethane, hexane and ethanol were used in these extraction processes [7, 18]. Alcoholic solvents such as methanol and ethanol were commonly used to prepare crude extract of *E. longifolia* [11, 39, 40]. This was because aquoues alcohol could recover a wide range of bioactive compounds from natural products [33]. In order to obtain terpenoids rich extract, further purification or fractionation

steps should be included as the crude extract is a complex mixture consisted of a vast group of bioactive compounds. The purification steps may involve liquid-liquid extraction (LLE), solid-liquid extraction such as column chromatography, solid phase extraction (SPE) and vacuum liquid chromatography (VLC) [8, 17].

LLE is a solvent partition technique which involves two immiscible liquids, namely sample in liquid and solvent as the extracting agent [31]. The phenomenon of mass transfer can occur during vigorous shaking of two immiscible liquids in LLE. A relatively less polar organic solvent is used to promote the mass transfer of less polar terpenoids into organic phase. For example, solvents like hexane, chloroform, ethyl acetate, dichloromethane, acetone, diethyl ether and butanol [5, 8, 12, 13, 17, 32]. On the other hand, terpenoid glycosides may remain in the aqueous phase, for instance, 70% ethanol, due to the presence of glycoside molecules [33-34]. Terpenoids containing ester bonds can easily be extracted by using strong polar solvent like 30% ethanol.

Solid phase extraction (SPE) can also be used to extract terpenoids with different molecule sizes or structures. Larger molecular terpenoids can be separated by using SPE in which it will be eluted out of the cartridge with longer retention time. Cyclic terpenoids with the same number of carbon will be eluted faster as the structure is more compact, and resulting a small sized molecule compared to a linear terpenoid [30].

Even though many studies have been conducted to extract terpenoids, the suitable and effective solvent to recover terpenoids from *E. longifolia* is not specifically reported till to date. The selection of solvent for a particular plant sample is likely varied according to the nature and composition of plant metabolites. Most of the studies were focused on crude extract, the reported technique for concentrating or purifying terpenoids is relatively limited in literature. Thus, it is important to investigate the most suitable solvent to recover terpenoids from the plant crude extract with the acceptable yield of terpenoid rich fraction. The objective of this study was to determine the suitable solvent for LLE in order to partition concentrated terpenoid rich fraction from its crude extract.

2. EXPERIMENTAL

2.1. Chemical and Plant Material

Acetic acid, oleanolic acid, vanillin and perchloric acid were bought from Fisher Scientific (Pittsburg, USA). Chlorofom, buthanol and ethyl acetate were of analytical grade were purchased from Merck (USA). Ultrapure water (18.2 megohm-cm) was produced from Barnstead NANOpure Diamond water purification system (State of Illinois, USA).

The roots of *E. longifolia* in the form of chips were provided by a local supplier from Pahang (Bentong, Malaysia). The samples were dried at 50°C in a drying oven (Memmert, Germany) until the moisture content was < 10% (Mettler Toledo HB43, USA). The root samples were ground and sieved into the size of 0.5- 1.0 mm. The ground samples of *E. longifolia* roots were stored at 4°C until further analysis [35].

2.2. Reflux Extraction

The method described by Kuo et al. [4] was used to obtain the crude extract *E. longifolia*. Three grams of ground *E. longifolia* was submerged in 300 mL of 70% ethanol and refluxed for 2 hours. The extract was filtered and concentrated by a vacuum rotary evaporator (Heidolph, Laborota 4000, Germany) at 50°C. The crude extract was dried in a drying oven (Memmert UN55, Germany) at 50°C and stored at -20°C before further analysis. The yield (%) of the crude extract was calculated based on the equation below:

$$\text{Yield of crude extract}\left(\%\frac{\text{w}}{\text{w}}\right)=\frac{\text{Mass of crude extract (g)}}{\text{mass of ground sample (g)}}\times 100\%$$

2.3. Liquid-Liquid Extraction of Terpenoids

The terpenoids of *E. longifolia* was partitioned using the method described by Kuo et al. [3]. The crude extract was reconstituted in water and partitioned using chloroform, butanol and ethyl acetate using LLE. The organic layer was collected and dried in a drying oven (Memmert UN55, Germany) at 50°C. The remaining aqueous layer was further extracted with the same and fresh solvent in twice. The organic layer was combined and dried to determine the yield of organic fraction. The remaining aqueous layer was also collected and dried in the oven. Both dried aqueous and organic phases were refrigerated at -20°C before further analysis. The yield (%) of the fractions was calculated based on the equation below:

$$\text{Yield of fraction}\left(\%\frac{\text{w}}{\text{w}}\right)=\frac{\text{Mass of dried fraction (g)}}{\text{Mass of crude extract (g)}}\times 100\%$$

2.4. Total Terpenoid Content

The total terpenoid content (TTC) was examined based on the method described by Chua et al. [35]. A 100 µL of 10 mg/L extract was re-dissolved in methanol. A 150 µL of 5% vanillin in glacial acetic acid and 500 µL perchloric acid were added to the extract. The mixture was incubated at 60°C for 45 minutes. The mixture was cooled to room

temperature, and consequently 2.5 mL glacial acetic acid was added. The samples were analysed using a UV-Vis spectrophotometer (UV 1800, Shimadzu, Kyoto, Japan) at 548 nm. Oleanolic acid (OA) was used as the positive control and a standard to obtain the standard calibration curve (30 – 1000 mg/L). A linear calibration curve was obtained ($R^2 > 0.99$) and the equation is as below:

$$Y = 0.001x + 0.2842$$

3. RESULTS AND DISCUSSION

3.1. Terpenoid Extraction

The crude extract of *E. longifolia* roots was prepared using a reflux system with 70% ethanol as the solvent. The use of polar solvent in the reflux process was to extract polar compounds such as terpenoid glycosides from *E. longifolia* roots [36-37]. From the experiments, the average yield of crude extract from *E. longifolia* roots was 5.70 ± 0.10%.

The dried crude extract was re-dissolved in water and further partitioned using LLE method with three different organic solvents. The organic solvents were ethyl acetate (EtAC), butanol (BuOH) and chloroform (CHCl3). A total of 6 fractions were produced, namely 3 organic fractions and another 3 aqueous fractions of their organic counterparts. The fractions were named as EtAC, BuOH, and CHCl3, and the remaining aqueous phases were EtAC-H2O, BuOH-H2O and CHCl3-H2O. Among the three solvents, CHCl3 fraction was the highest yield of 72.90 ± 0.15%, and followed by EtAC fraction, 52.80 ± 0.05%. On the other hand, BuOH fraction produced the lowest yield which was only 7.80 ± 0.04 %. The results are presented in Table 1.

Table 1 Total Yield of Organic and Aqueous Fractions using the Method of LLE

Organic Solvent	Yield (%w/w)	
	Organic	Aqueous
EtAC	52.80 ± 0.05	19.70 ± 0.07
BuOH	7.80 ± 0.04	52.70 ± 0.70
$CHCl_3$	72.90 ± 0.15	7.10 ± 0.07

3.2. Total Terpenoid Content of Organic and Aqueous Fractions

The TTC of all organic fractions are showed in Figure 1. It was found that $CHCl_3$ exhibited the highest TTC content which was 469.55 ± 7.04 mg OAE/L. Furthermore, BuOH and EtAC fractions showed to have 255.13 ± 8.99 and 313.30 ± 4.96 of mg OAE/L, respectively. It is concluded that CHCl3 is the most suitable solvent to isolate terpenoids from the crude extract of *E. longifolia* roots. The same result was also reported by Canbay [38], who found that $CHCl_3$ fractions showed to have high concentration of terpenoids. It was observed that EtAC-H_2O was the highest yield among the aqueous fractions which was 19.70 ± 0.07 %, and followed by BuOH-H_2O (52.7 ± 0.70%) and $CHCl_3$-H_2O (7.10 ± 0.07%).

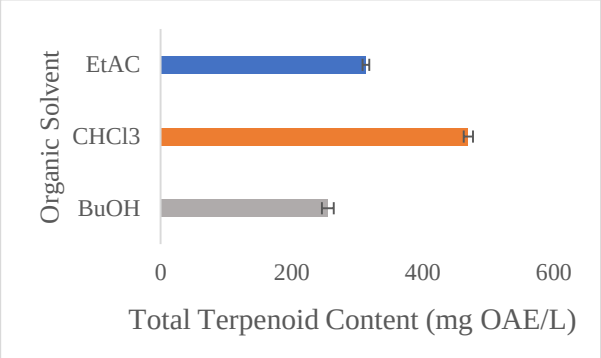

Figure 1 Total terpenoid content of organic fractions from LLE

The TTC of the aqueous phases is illustrated in Figure 2. The $CHCl_3$-H_2O showed the lowest terpenoids which was 1.16 ± 0.05 mg OAE/L, followed by EtAC-H_2O in 2.51 ± 0.16 mg OAE/L and BuOH-H_2O in 3.87 ± 0.24 mg OAE/L.

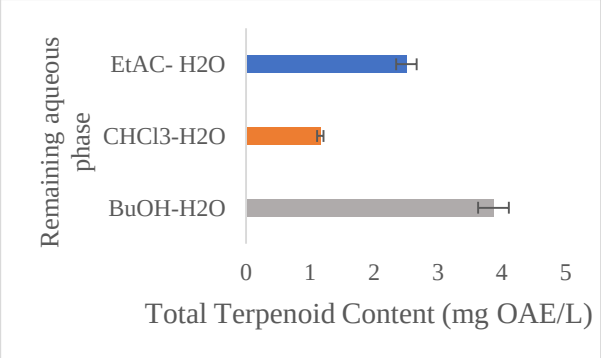

Figure 2 Total terpenoid content of remaining aqueous phases from LLE

It was observed that all aqueous fractions contained lower concentration of terpenoids than its organic counterparts. This suggests that solvent partition could be used to recover terpenoids from the crude extract. The yield or content of terpenoids is strongly depended on solvent polarity. The polarity of terpenoids that near to the polarity of solvent would be extracted and partitioned in the organic phase. While, the other compounds would be stayed in the remaining aqueous phase. This also explains that the remaining terpenoids in aqueous phases could have higher polarity. Terpenoids have been known to have a wide range of compounds with different polarities resulted from the presence of functional groups attached to the basic structure. $CHCl_3$ showed the highest yield of fraction and TTC content due to the presence of terpenoids or other similar polarity of compounds with chloroform in the *E. longifolia* roots [23].

4. CONCLUSION

This study was carried out to concentrate terpenoids from the crude extract of *E. longifolia* roots using three organic solvents such as EtAC, $CHCl_3$ and BuOH. Out of the three organic solvents, $CHCl_3$ fraction could recover the highest content of terpenoids (469.55 ± 0.01 mg OAE/L). This study proved that solvent partition using $CHCl_3$ in LLE could obtain a terpenoid rich fraction from *E. longifolia* roots. The terpenoid rich fraction is possibly used to increase the bioactivity of products formulated by the terpenoid rich fraction.

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